



STUDY OF THE EFFECTS OF HEAVY METALS ON THE BIOCHEMICAL CONSTITUENTS OF PROTEINS METABOLISM AND TOTAL NINHYDRIN POSITIVE SUBSTANCES USING ALTERNATE ANIMAL MODEL: HETEROMETRUS FULVIPES

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ABSTRACT

Heavy metals exposure in animals can lead to profound effects in growth and development. Heavy metals can target all protein metabolisms in the body, and are capable of disturbing most of the functions in animals where proteins are involved. Heavy metals primarily cause biochemical lesion and effects in altering protein metabolism. Chronic heavy metal exposure resulted in decrease in hepato-pancreatic weight, hepato-somatic index and embryonic length with subsequent reduction and length and weight of the embryos. An experimental study was performed with viviparous animal *Heterometrus fulvipes* to access the cumulative effects of these heavy metals on biochemical parameters of proteins, protein metabolism, and total ninhydrin positive substances (TNPS). Chronic exposure of sub lethal doses of heavy metals in *H. fulvipes*, depleted the protein content of the maternal tissues, hepatopancreas, pedipalps muscle and haemolymph. TNPS however was elevated with mercury exposure and decreased with lead. These changes can be attributed to possible proteotypic effect of the heavy metals to meet the excessive demands under the toxic stress. Therefore, it is necessary that heavy metal toxicity be well documented with further study in humans so that adequate precaution should be taken in mother and foetus to decrease its detrimental effects.

KEY WORDS: Heavy metals, Biochemical alteration, *Heterometrus fulvipes*, Protein, Injury.

INTRODUCTION:

Heavy metals are believed to cause primary biochemical alteration, protein being the most important biochemical substance can be profoundly affected. Proteins and amino acids are of paramount importance not only because of peculiar chemical and physiological properties but also because they appear to confer upon various types of cell and their biological specificity. Their main function is not primarily to supply energy but to furnish certain essential components of the tissues of the organism itself. Heavy metals induced protein depletion is seen in several animal species; *Bartelphusa guerini*, *Helix pomatia*, and *Anabas scandens* (1-3). It is, thus, clear from the literature that heavy metals, in general, affect the metabolism of animals. Administration of sodium chromate and calcium chromate to rats resulted in some significant changes in levels of total plasma protein (4). Administration of cadmium chloride to male Sprague-Dawley rats significantly increased serum proteins (5). Cadmium produced significant reduction in the protein content of *Daphnia magna* (6).

Heavy metals like mercury, lead and cadmium inhibit the amino acid transport (7). Exposure of seven human males, aged 35-42 years to industrial lead caused proximal tubular abnormalities i.e., aminoaciduria, glycosuria, phosphaturia stated that exposure of fish to mercury resulted in impaired carbohydrate metabolism and there was a trend in favour of protein utilization (8,9). Lead induced malabsorption of amino acids from intestine may also be one of the causative factors for the reduced level of proteins. Lead inhibits most of the enzymes by binding sulphhydryl group. So the depletion of protein could be attributed to the inhibition of enzymes involved in protein synthesis. Mercury (Hg) is a metal without biological function and an environmental pollutant frequently mentioned for causing toxic effects in living organisms (10). Its release can occur through natural sources such as volcanic activities or anthropogenic activities such as industrial processes, agriculture and mining, consequently making human exposure to Hg almost impossible to avoid (11,12). Mercury is the third most dangerous metal, right after arsenic and lead according to the Agency for Toxic Substance and Disease Registry (ATSDR) (13). Mercury can be found in two different forms: the organic form, which corresponds to a mercury atom bonded to carbon, and the inorganic form, which includes the elemental and oxidized forms (14). Elemental and organic mercury primarily affect the central nervous system (15, 16). Mercury chloride which is the object of this study is known to be a nephrotoxic agent (17, 18). It has been reported to cause disorders in the liver as well as in the reproductive and cardiovascular systems coupled with causing behavioural alterations (19-21). Exposure of *Channa punctatus* to sublethal concentration of mercury decreased the uptake of glycine (22). Similarly, exposure of *Sarotherodon mossambicus* to mercuric chloride decreased serum, muscle and liver proteins (23). Reduced incorporation of amino acids into protein was recorded during the symptomatic period of alkyl mercury poisoning (24).

The decline in the proteins was suggested to be a consequence of an intensive proteolysis, contributing to the increment of free amino acids fed into the Tricarboxylic acid cycle as keto acids (25). Excessive utilization of the reserve stores including proteins under the heavy metal stress is a consequence of metabolic impairment. The nutrient drain either due to metabolic impairment or to

meet the excessive energy demand resulting in negative balance in maternal animals, can interfere with the embryonic development.

Though, heavy metals are known to affect the protein metabolism of animals, actually very little is known as to how such effects exert their influence on the embryo in viviparous system. Heavy metals are known to cause adverse effects on the reproduction and development in mammals. There have been evidences of reduction in the embryonic size and failure of parturition of *H. fulvipes*, attributed to the inadequate supply of energy yielding substances to maintain the growth and development leading to successful parturition. In *H. fulvipes*, administration of a single to nine doses of mercury and lead caused reduction in the size of the embryos and even caused failure of parturition. (26) The extent, to which the possible impact of the heavy metals on the protein metabolism can be held responsible for the effects of mercury and lead on the embryonic development, is not known. Hence, a study of the impact of mercury and lead on the protein metabolism was undertaken by estimating the proteins and total ninhydrin positive substances (TNPS) in different maternal tissues and embryos following the administration of the heavy metals

MATERIAL AND METHODS:

Monthly samples for studying the effects of mercury and lead were drawn successively at intervals of one month from three groups divided on the basis of heavy metal exposure: groups I (control), group II (mercury exposure). Proteins and total ninhydrin positive substances were estimated in the samples drawn from Group I, Group II and Group III. Samples, drawn at each month from August to April, received one sub lethal dose every month and, hence, the sample obtained at August represented the effects of one sub lethal dose, whereas the samples at April reflected the combined effects of nine sub lethal doses of heavy metals.

Estimation of Proteins:

The tissue and haemolymph samples from *H. fulvipes* were homogenised in 5 ml of 5% TCA and centrifuged. The precipitate was dissolved in 1N sodium hydroxide. To this 0.2 ml of the protein solution, 5 ml of carbonate copper solution was added and allowed to stand for ten minutes. It was followed by the addition of 0.5 ml of diluted Folin reagent (1N) and after 30 minutes O.D. was read at 540 mμ. Protein contents of the samples were calculated from a standard graph plotted using Bovine serum albumin.

Estimation of TNPS:

The tissues and haemolymph samples were homogenised in 5.0% TCA and centrifuged. To 0.30 ml of the supernatant, 1.5 ml of Ninhydrin reagent was added and the solution was kept in boiling water bath for 5.5 minutes. The reaction mixture diluted to 10 ml using glass distilled water. O. D was read at 570 mμ. The TNPS of the samples were calculated from the standard graph using tyrosine. Colorimetric readings were taken using Bausch and Lomb Colorimeter (Spectronic-20). All the estimation was carried out between 9 am and 12 pm to avoid the possible influence of diurnal variation in *H. fulvipes*.

RESULTS:**Effect of mercury and lead on the protein and TNPS content of the hepatopancreas of the maternal animal:**

The protein content of the hepatopancreas of the control animals exhibits a pattern of variation during the gestation period increasing gradually till December followed by a decline thereafter up to March, and then a steady increase till May (Table-13, Fig-22). Similar patterns were visible with the gravid females, treated with sub lethal doses of mercury and lead. However, the protein content of the hepatopancreas was depressed significantly throughout the gestation period after the sub lethal doses of both lead and mercury. Though lead registered a higher degree of depressant action, it cannot be directly compared with mercury, as the doses were different, lead being $\frac{1}{3}$ LD50 of the value and mercury $\frac{1}{10}$ LD50.

Table 13: Effect of mercury (Hg) and lead (Pb) on the protein content of the hepato-pancreas of *H. fulvipes* during gestation period.

Values represent mean \pm S.E. Number of observations (N)=8.

Month of Treatment	protein mg/ 100mg wet wt.			
	control		Experimental	percent Change
AUG	15.15 \pm 0.43	Hg	13.53 \pm 0.74*	10.69
		Pb	13.01 \pm 0.76 ^a	14.12
SEPT	16.92 \pm 0.34	Hg	14.81 \pm 0.61 ^b	12.47
		Pb	14.22 \pm 0.68 ^b	15.95
OCT	16.35 \pm 0.54	Hg	14.15 \pm 0.56 ^a	13.45
		Pb	13.32 \pm 0.68 ^b	18.45
NOV	18.26 \pm 0.62	Hg	15.51 \pm 0.72 ^b	15.06
		Pb	14.98 \pm 0.72 ^b	17.96
DEC	16.68 \pm 0.39	Hg	14.64 \pm 0.71 ^b	13.31
		Pb	13.68 \pm 0.75 ^b	17.98
JAN	17.16 \pm 0.48	Hg	14.65 \pm 0.63 ^a	14.62
		Pb	13.86 \pm 0.57 ^c	19.23
FEB	14.82 \pm 0.77	Hg	12.71 \pm 0.54 ^a	14.23
		Pb	11.78 \pm 0.62 ^b	20.51
MAR	15.96 \pm 0.57	Hg	13.88 \pm 0.57 ^a	13.09
		Pb	13.38 \pm 0.53 ^a	16.16
APR	17.61 \pm 0.48	Hg	16.01 \pm 0.46 ^a	9.08
		Pb	15.78 \pm 0.75*	10.39

a_{ps}<0.05; b_p<0.01; c_p<0.00; *Insignificant

TNPS of the control animals followed a near reciprocal pattern compared to the pattern of variation of the proteins throughout the gestation period (Table-14; Fig-23). Administration of monthly sub lethal doses (1/10LD50 value) of mercury has elevated the TNPS, though insignificantly except of the later stages. Treatment with the sub lethal dose of the lead had an effect opposite to that of the mercury resulting in lowering of the free amino acid levels compared to the controls, though statistically not significant except during the last couple of months.

Table 14: Effect of mercury (Hg) and lead (Pb) on the protein content of the hepato-pancreas of *H. fulvipes* during gestation period.

Values represent mean \pm S.E. Number of observations (N)=8.

Month of Treatment	protein mg/ 100mg wet wt.			
	control		Experimental	percent Change
AUG	2.25 \pm 0.14	Hg	2.37 \pm 0.15*	5.55
		Pb	2.14 \pm 0.11 [†]	-4.66
SEPT	2.39 \pm 0.14	Hg	2.56 \pm 0.11 [†]	7.04
		Pb	2.10 \pm 0.10 [†]	-12.33
OCT	2.32 \pm 0.13	Hg	2.55 \pm 0.13 [†]	9.85
		Pb	2.05 \pm 0.19 [†]	-11.53
NOV	2.34 \pm 0.12	Hg	2.59 \pm 0.11 [†]	10.72
		Pb	2.03 \pm 0.18 [†]	-13.11
DEC	3.27 \pm 0.22	Hg	3.72 \pm 0.25 [†]	13.57
		Pb	2.85 \pm 0.10 [†]	-12.87
JAN	3.54 \pm 0.26	Hg	4.01 \pm 0.20 [†]	13.43
		Pb	3.05 \pm 0.25 [†]	-13.86
FEB	3.18 \pm 0.28	Hg	3.68 \pm 0.28 [†]	15.75
		Pb	2.72 \pm 0.19 [†]	-14.62
MAR	4.22 \pm 0.21	Hg	4.86 \pm 0.25 [†]	15.21
		Pb	3.49 \pm 0.26 [†]	17.23
APR	2.46 \pm 0.14	Hg	2.94 \pm 0.15 [†]	19.34
		Pb	1.92 \pm 0.10 ^b	-21.20

a_p<0.05; b_p<0.01; *Insignificant

Effect of mercury and lead on the protein and TNPS content of pedipalps muscle:

The protein content of the pedipalps muscle of the controls followed a declining course up to December followed by an elevation during rest of the gestation period. The same pattern of variation during the gestation period was obvious even in the pedipalps muscles of those animals treated with the heavy metals, mercury and lead. However, the treatment with sub lethal though the depletion was statistically significant beyond fourth dose administered for lead and beyond sixth dose for mercury (Table 15; fig.24). The pattern of variation in the TNPS content of the pedipalps muscles showed a near reciprocal relationship with the proteins (Table 16; Fig.25). The TNPS level was continuously elevated in the mercury treated animals indicating the natural consequence of depletion of proteins. On the contrary, lead administered resulted in the depletion of free amino acids in all samples tested during the gestation period.

Table 15: Effect of mercury (Hg) and lead (Pb) on the protein content of the pedipalpal muscle of *H. fulvipes* during the gestation period.

Values represent mean \pm S.E. Number of observations (N)=8.

Month of Treatment	protein mg/ 100mg wet wt.			
	control		Experimental	percent Change
AUG	14.13 \pm 0.57	Hg	13.35 \pm 0.60*	5.52
		Pb	13.28 \pm 0.54*	5.61
SEPT	13.10 \pm 0.60	Hg	12.11 \pm 0.46 [†]	7.55
		Pb	11.85 \pm 0.74 [†]	9.54
OCT	12.52 \pm 0.74	Hg	11.36 \pm 0.82 [†]	9.26
		Pb	11.23 \pm 0.65 [†]	10.30
NOV	12.45 \pm 0.55	Hg	10.98 \pm 0.44 ^a	11.80
		Pb	10.87 \pm 0.50 ^a	12.69
DEC	11.88 \pm 0.65	Hg	10.52 \pm 0.47 ^a	11.44
		Pb	9.97 \pm 0.40 ^a	16.07
JAN	13.25 \pm 0.61	Hg	11.65 \pm 0.65 ^a	12.07
		Pb	10.80 \pm 0.48 ^b	18.49
FEB	13.42 \pm 0.43	Hg	11.45 \pm 0.59 ^a	15.87
		Pb	11.25 \pm 0.61 ^b	17.34
MAR	13.15 \pm 0.49	Hg	11.51 \pm 0.40 ^a	12.47
		Pb	11.31 \pm 0.49 ^a	13.99
APR	13.90 \pm 0.46	Hg	12.20 \pm 0.48 ^a	12.23
		Pb	12.01 \pm 0.42 ^b	13.59

a_p<0.05; b_p<0.01; *Insignificant

Table 16: Effect of mercury (Hg) and lead (Pb) on the TPNS of the pedipalpal muscle of *H. fulvipes* during the gestation period.

Values represent mean \pm S.E. Number of observations (N)=8.

Month of Treatment	protein mg/ 100mg wet wt.			
	control		Experimental	percent Change
AUG	1.91 \pm 0.15	Hg	2.20 \pm 0.13*	14.80
		Pb	1.67 \pm 0.14 ^b	-12.87
SEPT	2.17 \pm 0.15	Hg	2.38 \pm 0.13 [†]	9.70
		Pb	1.96 \pm 0.11 ^b	-9.56
OCT	2.08 \pm 0.16	Hg	2.32 \pm 0.13 [†]	11.58
		Pb	1.93 \pm 0.10 ^a	-5.70
NOV	2.28 \pm 0.15	Hg	2.72 \pm 0.17 ^a	19.29
		Pb	2.25 \pm 0.12 ^a	-5.66
DEC	2.32 \pm 0.17	Hg	2.71 \pm 0.16 [†]	16.56
		Pb	2.16 \pm 0.10 ^a	-7.01
JAN	3.77 \pm 0.16	Hg	4.27 \pm 0.11 ^a	13.20
		Pb	3.43 \pm 0.17 ^b	-9.18
FEB	3.52 \pm 0.15	Hg	4.09 \pm 0.11 ^b	16.06
		Pb	3.06 \pm 0.10 ^b	-13.06
MAR	4.44 \pm 0.10	Hg	4.75 \pm 0.13 [†]	6.94
		Pb	3.91 \pm 0.12 ^b	-11.93
APR	2.57 \pm 0.17	Hg	2.90 \pm 0.12 ^a	13.10
		Pb	2.40 \pm 0.14 ^a	-6.41

a_p<0.05; b_p<0.01; *Insignificant

Effect of mercury and lead on protein and TNPS content of haemolymph:

The proteins in haemolymph of the gravid female gradually increased up to December (November samples) and then declined gradually up to May (April

samples) both in controls and in the animals treated with mercury or lead (Table-17; Fig-26). Though the pattern of variation was not altered, the heavy metals had a depressant action on the protein content becoming statistically significant subsequent to the administration of third dose. The hemolymph TNPS value in the controls and in the treated animals followed the same pattern of variation during the gestational period with a continuous increment from November up to march, having a reciprocal relationship with the haemolymph proteins (Table-18; Fig-27). Mercury elevated the free amino acids level, whereas lead had depleted the TNPS content of the haemolymph at all times during the gestational period.

Table 17: Effect of mercury (Hg) and lead (Pb) on the protein content of the hemolymph of *H. fulvipes* during the gestational period.

Values represent mean \pm S.E Number of observations (N)

Month of Treatment	protein g/ 100ml			
	control	Heavy metals	Experimental	Percent Depletion
AUG	4.87 \pm 0.28	Hg	4.61 \pm 0.21*	5.33
		Pb	4.49 \pm 0.22*	7.89
SEPT	6.78 \pm 0.31	Hg	6.15 \pm 0.24*	9.28
		Pb	6.01 \pm 0.25*	11.15
OCT	8.19 \pm 0.22	Hg	7.30 \pm 0.26a	10.87
		Pb	7.12 \pm 0.29b	13.01
NOV	9.00 \pm 0.22	Hg	8.41 \pm 0.26*	6.48
		Pb	7.96 \pm 0.18b	11.45
DEC	7.98 \pm 0.24	Hg	6.64 \pm 0.31b	16.82
		Pb	6.54 \pm 0.27c	18.12
JAN	6.80 \pm 0.25	Hg	5.99 \pm 0.32a	12.65
		Pb	5.80 \pm 0.25b	15.33
FEB	5.10 \pm 0.28	Hg	4.54 \pm 0.21*	10.91
		Pb	4.47 \pm 0.22*	12.38
MAR	5.11 \pm 0.13	Hg	4.30 \pm 0.20b	15.93
		Pb	4.37 \pm 0.14c	14.56

a_p<0.05; b_p<0.01; c_p<0.001; * insignificant

Table 18: Effect of mercury (Hg) and lead (Pb) on the NPS of the hemolymph of *H. fulvipes* during the gestational period.

Values represent mean \pm S.E. Number of observations (N)=8.

Month of Treatment	TNPS mg/100 ml		
	Control	Experimental	Percent Change
AUG	26.17 \pm 1.12	Hg 29.83 \pm 1.65a	11.77
		Pb 22.47 \pm 1.49a	-14.15
SEP	29.41 \pm 1.17	Hg 32.53 \pm 1.17	10.58
		Pb 25.28 \pm 1.05	-14.06
OCT	25.99 \pm 1.24	Hg 28.72 \pm 1.21*	10.49
		Pb 23.64 \pm 1.01	-9.03
NOV	27.63 \pm 1.12	Hg 30.88 \pm 1.01a	11.78
		Pb 25.18 \pm 1.09a	-8.84
DEC	30.90 \pm 1.02	Hg 33.25 \pm 1.19*	7.60
		Pb 27.16 \pm 1.58a	-12.09
JAN	31.44 \pm 1.52	Hg 35.31 \pm 1.11a	12.33
		Pb 27.97 \pm 1.08a	-11.01
FEB	31.72 \pm 1.29	Hg 34.79 \pm 1.65*	9.67
		Pb 27.63 \pm 1.15a	-12.88
MAR	33.45 \pm 1.12	Hg 36.17 \pm 1.01*	8.15
		Pb 30.55 \pm 1.19a	-8.64
APR.	30.17 \pm 1.14	Hg 33.11 \pm 1.24*	9.73
		Pb 26.14 \pm 1.14a	-13.37

a_p<0.05; * - insignificant

Effect of maternal treatment with mercury and lead on the protein and TNPS content of embryo:

The protein content of the embryos progressively increased during the gestational period concordance with the normal growth curve pattern, both in the controls and in the embryos of the treated animals. The heavy metals did not exert any statistically significant effect on the embryonic protein content, though there was a general decline in the proteins (Table 19; Fig- 28)

Both the controls and the groups with the heavy metal exposure showed a continuous increment throughout the period of the gestation in the TNPS content

of the embryos. The impact of the heavy metals on the protein metabolism was reflected in the depletion of free amino acids relative to controls by lead and elevation of the same by mercury (Table-20; Fig-29).

Table 19: Effect of maternal treatment with mercury (Hg) and lead (Pb) on the protein content of the embryos of *H. fulvipes* during the gestation period.

Values represent mean \pm S.E. Number of observations (N)=8.

Month of Treatment	Protein (mg) / embryo		
	Control	Experimental	Percent Change
AUG	0.03 \pm 0.01	Hg 0.03 \pm 0.02*	7.89
		Pb 0.03 \pm 0.02*	10.52
SEP	0.05 \pm 0.01	Hg 0.04 \pm 0.01*	9.43
		Pb 0.04 \pm 0.01*	13.20
OCT	0.06 \pm 0.01	Hg 0.06 \pm 0.01*	7.35
		Pb 0.06 \pm 0.01*	10.29
NOV	0.10 \pm 0.04	Hg 0.10 \pm 0.01*	3.70
		Pb 0.10 \pm 0.01*	7.40
DEC	0.11 \pm 0.04	Hg 0.11 \pm 0.02*	5.17
		Pb 0.10 \pm 0.02*	7.75
JAN	0.29 \pm 0.06	Hg 0.26 \pm 0.09*	7.56
		Pb 0.25 \pm 0.08*	13.74
FEB	0.31 \pm 0.09	Hg 0.27 \pm 0.09*	13.60
		Pb 0.26 \pm 0.09*	17.72
MAR	0.75 \pm 0.09	Hg 0.70 \pm 0.08*	6.10
		Pb 0.69 \pm 0.08*	7.43
APR.	2.24 \pm 0.63	Hg 2.13 \pm 0.64*	4.81
		Pb 1.99 \pm 0.50*	11.19

* Insignificant

Table 20: Effect of maternal treatment with mercury (Hg) and lead (Pb) on the TNPS of the embryos *H. fulvipes* during the gestational period.

Values represent mean \pm S.E number of observations (N)=10.

Month of Treatment	TNPS (mg) / embryo		
	Control	Experimental	Percent Change
AUG	0.04 \pm 0.005	Hg 0.05 \pm 0.006*	14.58
		Pb 0.04 \pm 0.005*	-12.50
SEP	0.07 \pm 0.006	Hg 0.08 \pm 0.003*	16.21
		Pb 0.06 \pm 0.006*	-10.81
OCT	0.10 \pm 0.008	Hg 0.12 \pm 0.006*	13.20
		Pb 0.08 \pm 0.004*	-17.92
NOV	0.11 \pm 0.008	Hg 0.12 \pm 0.008*	8.54
		Pb 0.10 \pm 0.006*	-7.69
DEC	0.40 \pm 0.03	Hg 0.44 \pm 0.02*	10.89
		Pb 0.34 \pm 0.03*	-14.85
JAN	0.58 \pm 0.03	Hg 0.63 \pm 0.02*	8.04
		Pb 0.54 \pm 0.03*	-6.33
FEB	0.60 \pm 0.01	Hg 0.62 \pm 0.02*	15.89
		Pb 0.55 \pm 0.04*	-8.26
MAR	0.86 \pm 0.03	Hg 1.01 \pm 0.02*	16.62
		Pb 0.73 \pm 0.04*	-15.24
APR	1.36 \pm 0.11	Hg 1.44 \pm 0.12*	5.56
		Pb 1.27 \pm 0.11a	-6.73

a_p<0.05; b_p<0.01; c_p<0.001; * insignificant

DISCUSSION:

In the present study, the heavy metals mercury and lead, administered in the sub lethal doses to *H. fulvipes* depleted the protein content of the maternal tissues, hepatopancreas, pedipalps muscle and hemolymph, suggesting a proteolytic effect of the heavy metals possibly to meet the excessive demands under the toxic stress. The TNPS free amino acids showing an elevation following the administration of mercuric chloride can be considered to bear a testimony to proteolysis as a consequence of which the levels of amino acids rise. Even though the elevation of amino acids can also be a consequence of impairment of incorporation of amino acids into proteins the concomitant reductions in proteins support the view that mercury exerts a proteolytic effect. Sub lethal dose of lead while exerting a proteolytic effect on the maternal tissues of *H. fulvipes* depleted the TNPS contrary to the effect observed on administration of mercuric chloride. The depletion of TNPS in all the tissues concomitant with the depletion of proteins following the administration of lead, would suggest the utilization of free amino acids for release of energy through TCA cycle to meet the excessive energy demands imposed by the toxic stress of lead. The opposite effects observed in response to sub lethal doses of lead and mercury on the TNPS levels

in the tissues of *H. fulvipes* perhaps has a bearing on the difference in the dose administered, mercury being 1/10th and lead being 1/3rd the LD50 value, apart from the impact of metals themselves.

The effects of both lead and mercury on the proteins and TNPS levels in the maternal tissues and embryos of *H. fulvipes* do not exhibit an ever increasing difference between the controls and experimental animals proportional to the increase in the number of doses of the heavy metals administered. The absence of a very conspicuous dose dependent proportionality of a change in the proteins and TNPS, perhaps suggests a fairly quick unloading of heavy metal burdens preventing the cumulative effect. There are reported incidences of a similar reduction (though statistically not significant) in the protein content of the embryos of *H. fulvipes* following the administration of lead and mercury, which can be attributed to the reduced supply of proteins by the mother because of depleted levels in maternal tissues, or because of the inability of the embryos to draw proteins from the mother to the extent of maintaining the proteins levels, or because of the lowered synthesis and incorporation of embryonic proteins owing to heavy metal toxicity. As the main function of proteins and amino acids is to furnish certain essential components during tissue formation, the depleted proteins might affect the formation of various tissues in embryos resulting in failure of parturition. Other notable direct action of heavy metals on the embryos with various effects were lowered accumulation of proteins, decreased size of embryos and pulleys, and failures of parturition.

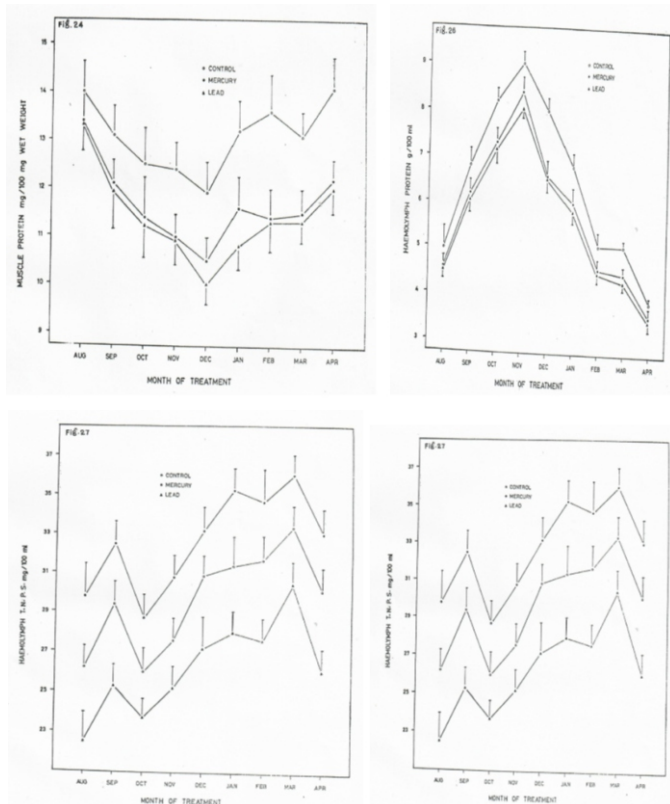
CONCLUSION:

Heavy metals are known to exert significant changes in the biochemical constituents of many animals. Proteins, being vital biochemical constituents for growth and development, are directly affected with subsequent exposure of mercury and lead. This has been shown with the administration of sub lethal doses of heavy metals in *H. fulvipes*, which depleted the protein content of the maternal tissues, hepatopancreas, pedipalps muscle and haemolymph. TNPS however was elevated with mercury exposure and decreased with lead. These effects of the heavy metals can be attributed to the excessive demands under the toxic stress and require further study to demonstrate the possible effect in higher animals including humans.

Conflict of Interest: None

Disclosure: None

FIGURE:



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